(vii) selecting offspring of (vi) by the presence of a detectable indicator resulting from both the first and second indicator components in tissue or specialized cells of the offspring.

REMARKS

Claims 1, 3-6, 9, 12, 13 and 15-19 are currently pending and presented for examination. Claims 1, 6, 12, 13, 18 and 19 have been amended. Support for the amendments to the claims is found throughout the specification as originally filed. More particularly, support for the amendment to claim 12 is found, *inter alia*, on page 16, lines 10-12 of the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made."

The Invention

The present invention relates to gene entrapment vectors and their use in gene discovery, and their use in screening for or making cells and organisms that are mutated for such genes. This invention also relates to the use of such entrapment vectors to identify tissue specific transcription control elements such as promoters and enhancers and for generating transgenic animals displaying restricted expression of transgenes. This invention also relates to trap vectors comprising a splice acceptor and a sequence encoding a reporter gene.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 1, 3-6, 12, 13 and 16-19 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly not being enabling for the use of ES cells from an organism other than a mouse. Applicants respectfully point out that the reference to claims 9 and 15 as being rejected under 35 U.S.C. § 112 is presumably an error. The Examiner has indicated that claims 9 and 15 are allowable, and these claims do not refer to ES cells of

any type. To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection. Claims 1, 13, 18 and 19 have been amended to substitute the word "mouse" for each instance of the word "murine", as suggested by the Examiner. Furthermore, claim 6 has been amended to be consistent with claim 1, and claim 19 has been amended to make it internally consistent. In view of the amendments to the claims, Applicants respectfully request that the rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

Rejection under 35 U.S.C. § 102(e)

Claims 12 and 13 were rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by U.S. Patent No. 6,207,371 ("Zambrowicz *et al.*"). According to the Office Action, Zambrowicz *et al.* teach a vector comprising the combination of a 3' exon trap and a promoter trap, each of which could separately be used for integration into the genome of a eukaryotic cell. The 3' exon trap comprises a promoter operably linked to a selectable marker. The promoter trap comprises in the 5' to 3' direction, a splice acceptor, a selectable marker, and an IRES element. The selectable markers are allegedly equivalent to the indicator components of the instant claims. To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

> To anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter.
>
> *PPG Industries Inc. v. Guardian Industries Corp.*, 37 USPQ2d.

Zambrowicz *et al.* disclose vectors for the construction of a library of mutated cells (*see*, Abstract, Zambrowicz *et al.*). These vectors for inserting foreign exons into animal cell transcripts comprise a selectable marker, a promoter element operatively positioned 5' to the selectable marker, a splice donor site operatively positioned 3' to the selectable marker, and a second mutagenic foreign polynucleotide

sequence located upstream from the promoter element that disrupts the splicing or read-through expression of the endogenous cellular transcript (*see*, column 3, lines 2-10 of Zambrowicz *et al.*).

The Office Action alleges that VICTR20 comprises a selectable maker equivalent to the indicator components of the instant claims. VICTR20 provides two potential positive selectable markers (puro and neo) (*see*, column 11, lines 27-28, Zambrowicz *et al.*). Either puromycin alone or neomycin alone is able to function as a selectable marker. Both markers need not be present to achieve a detectable signal; a single selectable marker is fully capable of producing a detectable signal. At no point do Zambrowicz *et al.* teach or suggest the use of indicator components.

Claims 12 of the present invention reads as follows:

12. (Twice Amended) The combination of:

(i) a DNA construct for integration into the genome of an eukaryotic cell comprising a sequence encoding a ***first indicator component*** under the control of a promoter having restricted expression; and

(ii) a DNA construct for integration into the genome of a eukaryotic cell, comprising in the 5' to 3' direction, a splice acceptor, a sequence encoding a ***second indicator component*** not operably linked to a transcription control element, and an optional IRES, wherein expression of ***both the first and second indicator components in said cell is detectable***, and wherein ***in the absence of either indicator component, there is no detectable indicator.***

Contrary to what is stated in the Office Action, the selectable markers of the VICTR vectors described by Zambrowicz *et al.* are ***not*** equivalent to the indicator components of the present invention. The "detectable indicator" of the present invention is a detectable event, which results from the expression of ***more than one indicator component*** in a cell (*see*, page 16, lines 10-12 of the specification). The detectable

indicator results from ***enzymatic complementation*** of proteins or peptides expressed from the first and second indicator component coding sequences (*see*, page 20, lines 28-33 of the specification). The present invention may be carried out using any detectable gene products, which exhibit intra-cistronic complementation, including, for example, alpha and omega fragments of β-gal. An alpha or omega fragment of β-gal in the absence of the complementary fragment is ***not*** a detectable indicator and cannot be used as a selectable marker. Thus, in stark contrast to Zambrowicz *et al.*, a single indicator component alone is ***not*** sufficient to produce a detectable indictor or selectable marker. In order to produce a detectable indicator, more than one indicator component must be expressed in a cell. Therefore, a signal will only be detectable if both components are expressed, indicating that the gene is transcribed in the tissue of interest.

The Office Action points to column 14, lines 51-60 of Zambrowicz *et al.* as allegedly teaching "tissue restricted promoters". Contrary to what is stated in the Office Action, this passage in Zambrowicz *et al.* only teach the use of "inducible regulatory elements", which may be distinguished from "tissue restricted promoters" as follows. The inducement taught in Zambrowicz *et al.* is ***exogenous***, and is not tissue or cell type restricted as taught by the present application. In the present application, the inducement of the promoter is ***endogenous***, because the promoter is induced by a tissue specific or cell specific endogenous inducing element. Both No *et al.* and Furth *et al.* (*see*, column 14, lines 54-56 of Zambrowicz *et al.*) teach ***exogenously administered inducing elements***, where the elements ecdysone and tetracycline respectively are not native to the transgenic mice and therefore must be administered exogenously. The present invention teaches tissue or cell specific promoters endogenous to the mouse, which are capable of inducement by ***endogenously expressed inducing elements*** (*see*, page 13, lines 13-28 of the specification). Therefore, these promoters are only induced in the cell or tissue type in which the promoter is functional. In this case, the tissue type or cell type itself signals the identity of the tissue or cell. Thus, Zambrowicz *et al.* do ***not*** teach the use of such promoters having restricted expression as is taught by the present

application. As such, Zambrowicz *et al.* do ***not*** disclose every element of the challenged claim.

Zambrowicz *et al.* do ***not*** teach indicator components that function by enzymatic complementation. Furthermore, as discussed above, Zambrowicz *et al.* teach selectable markers which are fully capable of producing a detectable indicator even when they are expressed in the absence of any other marker, for example, βgeo, neomycin, or puromycin (*see*, column 7, lines 19-22, Zambrowicz *et al.*). βgeo, neomycin, or puromycin are commonly used as selectable markers, and it is well known in the art that each produces a detectable signal on its own. In addition, Zambrowicz *et al.* do ***not*** teach the use of tissue restricted promoters. Therefore, Zambrowicz *et al.* do ***not*** anticipate the present invention. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 102(e) be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (925) 472-5000.

X 2002

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claims 1, 13 and 18 have been amended in the following manner:

1. (Twice Amended) A method of screening for the integration of a DNA construct into a target gene having restricted expression in a mouse, said method comprising:

(i) transforming a [murine] mouse ES cell with a first DNA construct encoding a first indicator component under the control of a promoter having restricted expression in a mouse;

(ii) transforming the cell of (i) or a descendent of the cell by operably integrating into the cell's genome, a second DNA construct comprising DNA encoding a second indicator component not operably linked to a transcription control element;

(iii) producing tissue or specialized cells from the cell of (ii); and

(iv) monitoring the tissue or specialized cells of (iii) for a detectable indicator resulting from both the first and second indicator components indicative of integration of the second DNA construct into a gene having restricted expression.

6. (Twice Amended) The method of claim 1 which additionally comprises isolating DNA endogenous to the mouse ES cell or descendent thereof which flanks the second DNA construct integrated into a gene having restricted expression.

12. (Twice Amended) The combination of:

(i) a DNA construct for integration into the genome of an eukaryotic cell comprising a sequence encoding a first indicator component under the control of a promoter having restricted expression; and

(ii) a DNA construct for integration into the genome of a eukaryotic cell, comprising in the 5' to 3' direction, a splice acceptor, a sequence encoding a second indicator component not operably linked to a transcription control element, and an optional IRES, wherein expression of both the first and second indicator components in said cell is detectable, and wherein in the absence of either indicator component, there is no detectable indicator.

13. (Twice Amended) A [murine] mouse ES cell or descendent thereof, transformed by the combination of DNA constructs of claim 12.

18. (Twice Amended) A method of producing [murine] mouse tissue or specialized cells comprising a detectable indicator associated with a target gene having restricted expression, which comprises:

(i) transforming a [murine] mouse ES cell with a first DNA construct encoding a first indicator component under the control of a promoter having restricted expression in a mouse;

(ii) transforming the cell of (i) or a descendent of the cell by integrating into the cell's genome, a second DNA construct comprising DNA encoding a second indicator component not operably linked to a transcription control element;

(iii) producing tissue or specialized cells from the cell of (ii); and

(iv) selecting tissue or specialized cells of (iii) by the presence of a detectable indicator resulting from both the first and second indicator components.

19. (Twice Amended) A method of producing a mouse comprising a detectable indicator associated with a target gene having restricted expression, which comprises:

(i) transforming a [murine] mouse ES cell by integrating into the cell's genome, a first DNA construct encoding a first indicator component under the control of a promoter having restricted expression;

(ii) transforming the cell of (i) or a descendent of the cell by integrating into the cell's genome, a second DNA construct comprising DNA encoding a second indicator component not operably linked to a transcription control element;

(iii) selecting transformed cells of (ii);

(iv) introducing selected cells of (iii) into a [murine or porcine] mouse host embryo;

(v) implanting the host embryo of (iv) into a pseudopregnant [mammal] mouse;

(vi) maintaining the [mammal] mouse of (v) while offspring develops to term from the host embryo; and

(vii) selecting offspring of (vi) by the presence of a detectable indicator resulting from both the first and second indicator components in tissue or specialized cells of the offspring.